Cypate-Gly-Arg-Asp-Ser-Pro-Lys
Cyp-GRD

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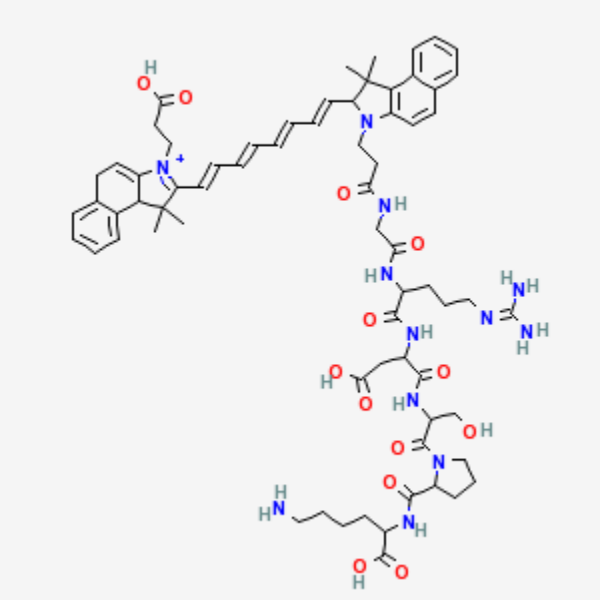
Chemical name:	Cypate-Gly-Arg-Asp-Ser-Pro-Lys
Abbreviated name:	Cyp-GRD, Cyp-GRDSPK
Synonym:	
Backbone:	Peptide
Target:	$\alpha_v\beta_3$ integrin
Mechanism:	Receptor binding
Method of detection:	Optical, near-infrared fluorescence
Source of signal:	Cypate
Activation:	No
***In vitro* studies:**	Yes
Rodent studies:	Yes
Other non-primate mammal studies:	No
Non-human primate studies:	No
Human studies:	No



Click on the above structure for additional information in PubChem [http://pubchem.ncbi.nlm.nih.gov/].

Background

[PubMed]

Integrins are a family of cell surface heterodimeric glycoproteins that mediate diverse biological events involving cell-cell and cell-matrix interactions (1). They consist of an α and a β subunit. They are important for cell adhesion and signal transduction. The $\alpha_v\beta_3$ integrin is the most prominent receptor class affecting tumor growth, tumor invasiveness, metastasis, tumor-induced angiogenesis, inflammation, osteoporosis, and rheumatoid arthritis (2-7). The $\alpha_v\beta_3$ integrin is strongly expressed on tumor cells and activated endothelial cells (3). In contrast, expression of $\alpha_v\beta_3$ integrin is weak on resting endothelial cells and most normal tissues. The $\alpha_v\beta_3$ antagonists are being studied as anti-tumor and anti-angiogenic agents (4, 8, 9), and the agonists are being studied as angiogenic agents for coronary angiogenesis (10, 11). A tripeptide sequence consisting of Arg-Gly-Asp (RGD)

is identified as a recognition motif used by extracellular matrix proteins (vitronectin, fibrinogen, laminin, and collagen) to bind to a variety of integrins including $\alpha_v\beta_3$. Various radiolabeled antagonists and peptides were introduced for imaging of tumors and tumor angiogenesis (12).

Optical fluorescence imaging is increasingly used to obtain biological functions of specific targets (13, 14). However, the intrinsic fluorescence of biomolecules poses a problem when visible light (350-700 nm) absorbing fluorophores are used. Near-infrared (NIR) fluorescence (700-1000 nm) detection avoids the background fluorescence interference of natural biomolecules, providing high contrast between target and background tissues. NIR fluorophores have wider dynamic range and minimal background as a result of reduced scattering compared with visible fluorescence detection. They also have high sensitivity, resulting from low infrared background, and high extinction coefficients, which provide high quantum yields. The NIR region is also compatible with solid-state optical components, such as diode lasers and silicon detectors. NIR fluorescence imaging is becoming a noninvasive alternative to radionuclide imaging.

Cypate is a reactive carbocyanine dye, which is derived from indocyanine green (ICG) (15). Cypate was previously conjugated to octreotate (Cyp-OC). Cyp-OC was not toxic to rats up to 10 μmol/kg (16). From the results of investigating a small library of RGD peptides for their binding activity to the $\alpha_v\beta_3$ integrin, a linear hexapeptide, Gly-Arg-Asp-Ser-Pro-Lys (GRDSPK), lacking the RGD sequence was conjugated with Cypate as Cyp-GRD to study *in vivo* biodistribution of the tracer in tumor-bearing mice (17). Cypate is a NIR fluorescent dye with an absorbance maximum at 778 nm and an emission maximum at 805 nm with a high extinction coefficient of 224,000 $(mol/L)^{-1}cm^{-1}$. Cyp-GRD was found to have a high and long-lasting accumulation in $\alpha_v\beta_3$-positve A549 human non-small cell lung carcinomas in nude mice. The binding of Cyp-GRD to the integrin receptor was found to be specific both *in vitro* and *in vivo*.

Synthesis

[PubMed]

A detailed synthesis of Cypate was reported by Ye et al. (15). Cypate was conjugated to peptides prepared on solid support by standard fluorenylmethoxycarbonyl peptide synthesis (17). The conjugated peptides were subsequently cleaved from the solid resin and purified by high-performance liquid chromatography (HPLC) in good yields and high purity (>95%).

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

A549 cells were validated to have α_v and β_3 integrins by immunohistochemistry and Western blot analysis (17). The β_3 subunit was expressed at a higher level than the α_v subunit, suggesting that β_3 may also be associated with another α subunit.

Fluorescence microscopy was used to study cellular distribution of Cyp-GRD in A549 cells (17). Cyp-GRD (1 μM) was internalized into the cytoplasm and not the nucleus by the cells at 37° C after 30 min of incubation, whereas the tracer remained on the cell surface at 4° C. The binding of the Cyp-GRD was inhibited by 10 μM cyclo(Arg-Gly-Asp-D-Phe-Val), an RGD antagonist with little

fluorescence that was visible in A549 cells. The binding and internalization of Cyp-GRD were also inhibited by anti-β_3 antibody (10 μM) and to a lesser extent by anti-α_v antibody (10 μM), suggesting an important role for the β_3 subunit in the binding and internalization of Cyp-GRD in A549 cells. Cyp-GRD was not toxic to A549 cells up to 100 μM as measured by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay.

Animal Studies

Rodents

[PubMed]

Biodistribution studies of Cyp-GRD were evaluated in nude mice bearing an A549 subcutaneous xenograft model (17). Whole-body small animal images by reflectance planar fluorescence were obtained at various time points after injection of Cyp-GRD (0.3 μmol/kg). The tumor uptake of Cyp-GRD was visible at 8 h after the tracer had been cleared from the blood and non-targeted tissues. A maximal uptake in the tumor was reached at 24 h. The tracer uptake in the tumor could be blocked (>80%) by co-injection of cyclo(Arg-Gly-Asp-D-Phe-Val) (0.3 μmol/kg), whereas the blocking resulted in higher fluorescence intensity in the liver and kidneys. Use of an optical technique provided noninvasive imaging of tumor cells in mice.

The biodistribution of Cyp-GRD, Cypate-Gly-Arg-Gly-Asp-Ser-Pro-Lys (Cyp-RGD), and Cypate-cyclo(Arg-Gly-Asp-D-Phe-Val-Lys) (Cyp-cyclo-RGD) was studied in A549 tumor-bearing mice by *ex vivo* tissue fluorescence intensity measurements at 24 h after injection (17). Cyp-GRD uptake in tumor was the highest, followed to a lesser extent by Cyp-cyclo-RGD. The uptake of Cyp-RGD in the tumor was minimal. Cyp-cyclo-RGD and Cyp-RGD were markedly retained in the liver and kidneys as compared with Cyp-GRD. Cyp-GRD had a minimal uptake in non-targeted tissues. Furthermore, ^{111}In-DOTA-GRD was not retained by the tumor. Therefore, there seems to be a synergic effect of Cypate- and GRD-containing peptide for binding to the $\alpha_v\beta_3$ integrin.

Other Non-Primate Mammals

[PubMed]

No publication is currently available.

Non-Human Primates

[PubMed]

No publication is currently available.

Human Studies

[PubMed]

No publication is currently available.

References

1. Hynes RO, Lively JC, McCarty JH, Taverna D, Francis SE, Hodivala-Dilke K, Xiao Q. The diverse roles of integrins and their ligands in angiogenesis. Cold Spring Harb Symp Quant Biol 67:143–153; 2002. (PubMed)
2. Grzesik WJ. Integrins and bone--cell adhesion and beyond. Arch Immunol Ther Exp (Warsz) 45:271–275; 1997. (PubMed)
3. Jin H, Varner J. Integrins: roles in cancer development and as treatment targets. Br J Cancer 90:561–565; 2004. (PubMed)
4. Kumar CC. Integrin alpha v beta 3 as a therapeutic target for blocking tumor-induced angiogenesis. Curr Drug Targets 4:123–131; 2003. (PubMed)
5. Ruegg C, Dormond O, Foletti A. Suppression of tumor angiogenesis through the inhibition of integrin function and signaling in endothelial cells: which side to target? Endothelium 9:151–160; 2002. (PubMed)
6. Varner JA, Cheresh DA. Tumor angiogenesis and the role of vascular cell integrin alphavbeta3. Important Adv Oncol □□□:69–87; 1996. (PubMed)
7. Wilder RL. Integrin alpha V beta 3 as a target for treatment of rheumatoid arthritis and related rheumatic diseases. Ann Rheum Dis 61:ii96–ii99; 2002. (PubMed)
8. Kerr JS, Mousa SA, Slee AM. Alpha(v)beta(3) integrin in angiogenesis and restenosis. Drug News Perspect 14:143–150; 2001. (PubMed)
9. Mousa SA. alphav Vitronectin receptors in vascular-mediated disorders. Med Res Rev 23:190–199; 2003. (PubMed)
10. Sadeghi MM, Krassilnikova S, Zhang J, Gharaei AA, Fassaei HR, Esmailzadeh L, Kooshkabadi A, Edwards S, Yalamanchili P, Harris TD, et al. Detection of injury-induced vascular remodeling by targeting activated alphavbeta3 integrin in vivo. Circulation 110:84–90; 2004. (PubMed)
11. Sinusas AJ. Imaging of angiogenesis. J Nucl Cardiol 11:617–633; 2004. (PubMed)
12. Haubner R, Wester HJ. Radiolabeled tracers for imaging of tumor angiogenesis and evaluation of anti-angiogenic therapies. Curr Pharm Des 10:1439–1455; 2004. (PubMed)
13. Ntziachristos V, Bremer C, Weissleder R. Fluorescence imaging with near-infrared light: new technological advances that enable in vivo molecular imaging. Eur Radiol 13:195–208; 2003. (PubMed)
14. Achilefu S. Lighting up tumors with receptor-specific optical molecular probes. Technol Cancer Res Treat 3:393–409; 2004. (PubMed)
15. Ye Y, Bloch S, Kao J, Achilefu S. Multivalent carbocyanine molecular probes: synthesis and applications. Bioconjug Chem 16:51–61; 2005. (PubMed)
16. Achilefu S, Dorshow RB, Bugaj JE, Rajagopalan R. Novel receptor-targeted fluorescent contrast agents for in vivo tumor imaging. Invest Radiol 35:479–485; 2000. (PubMed)
17. Achilefu S, Bloch S, Markiewicz MA, Zhong T, Ye Y, Dorshow RB, Chance B, Liang K. Synergistic effects of light-emitting probes and peptides for targeting and monitoring integrin expression. Proc Natl Acad Sci U S A 102:7976–7981; 2005. (PubMed)